IN THE UNITED STATES PATENT OFFICE

In re application of

Florian Thaler, et al.

Serial No. ***10/579,675*** Group Art Unit ***1626***

Filed: ***May 18, 2006*** Examiner: ***NOLAN, Jason Michael***

For: ***"3-AMINOPYRROLIDONE DERIVATIVES"***

DECLARATION UNDER RULE 132

I, Patricia Salvati, am a citizen of Italy and reside at Via Valera, 16/C, 20020 ARESE (Milano), Italy.

I obtained a doctorate degree with honors in Biological Sciences from the University of Bologna; I underwent post doctoral training in Pharmacology at the University of Pavia, followed by additional training at the University College, London, UK; Prostaglandin Unit, Wellcome Research Lab. Beckenham, Kent, U.K.; New York Medical College, Valhalla, US; Biophysics Institute, Aarhus University Denmark; Shimane University, Izumo, Japan.

Having gained extensive experience first in GI pharmacology, then in cardiovascular research, as of 1993 my research has been fully devoted to Neuropharmacology.

I am the author of over 60 patents and over 90 publications.

I have extensive experience in leading drug development projects in the Industry. In 1978 I joined Farmitalia Carlo Erba where I became Head of Cardiovascular Pharmacology and then Director of Cardiovascular Research in 1990. I was appointed

Head of CNS Pharmacology and Project Leader of the Antiepileptic project in Pharmacia & Upjohn in 1995.

In 1998 I was one of the 3 founders of Newron Pharmaceuticals S.p.A. where I was holding the position of Head of Discovery till 2007. In 2008 I was appointed Head of Preclinical Research and Development.

In this capacity and as inventor of the application in re, I

DECLARE

I was requested to carry out a study on Inhibition of N-Type calcium channels by some representative compounds of the invention as per re., in comparison with the reference standard ralfinamide. In vitro results are shown in Table 1.

For the method see Example 10 "Calcium Influx assay" of the specification.

Table 1

Compounds	Ca N-type IC50 μM
(S)-3-[3-Methyl-4-(naphthalen-1-ylmethoxy)-benzylamino]-pyrrolidin-2-one	8.2
(S)-3-[3-Methyl-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	8.8
(S)-3-[3-Bromo-5-methoxy-4-(naphthalen-1-ylmethoxy)-benzylamino]-pyrrolidin-2-one	13.8
(S)-3-[3-Bromo-4-(3,5-dimethoxy-benzyloxy)-benzylamino]-pyrrolidin-2-one	13.3
(S)-3-[4-(3,4-Dichloro-benzyloxy)-3-fluoro-benzylamino]-pyrrolidin-2-one	6.7
(S)-3-[3-Fluoro-4-(naphthalen-1-ylmethoxy)-benzylamino]-pyrrolidin-2-one	5.0
(S)-3-[3-Fluoro-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	3.2
(S)-3-[3-Fluoro-4-(4-trifluoromethyl-benzyloxy)-benzylamino]-pyrrolidin-2-one	9.6
(S)-3-[3-Fluoro-4-(2-phenoxy-ethoxy)-benzylamino]-pyrrolidin-2-one	5.7
(S)-3-(4-Benzyloxy-2-chloro-benzylamino)-pyrrolidin-2-one	2.7
(S)-3-[2-Chloro-4-((E)-3-phenyl-allyloxy)-benzylamino]-pyrrolidin-2-one	4.2
(S)-3-[2-Chloro-4-(3,4-dichloro-benzyloxy)-benzylamino]-pyrrolidin-2-one	9.0
(S)-3-[2-Chloro-4-(naphthalen-1-ylmethoxy)-benzylamino]-pyrrolidin-2-one	9.5
(S)-3-[2-Chloro-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	7.0
(S)-3-[2-Chloro-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	7.3
(S)-3-[3-Bromo-5-methoxy-4-(4-trifluoromethyl-benzyloxy)-benzylamino]-pyrrolidin-2-one	12.4
(S)-3-[3,5-Dimethoxy-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	3.4
(S)-3-(4-Benzyloxy-3,5-dimethyl-benzylamino)-pyrrolidin-2-one	13.0
(S)-3-[3,5-Dimethyl-4-((E)-3-phenyl-allyloxy)-benzylamino]-pyrrolidin-2-one	9.5
(S)-3-(3,5-Dimethyl-4-pentyloxy-benzylamino)-pyrrolidin-2-one	13.9
(S)-3-[4-(3,4-Dichloro-benzyloxy)-3,5-dimethyl-benzylamino]-pyrrolidin-2-one	4.8
(S)-3-[3,5-Dimethyl-4-(naphthalen-1-ylmethoxy)-benzylamino]-pyrrolidin-2-one	11.3
(S)-3-[3,5-Dimethyl-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	11.4
(S)-3-[3,5-Dimethyl-4-(4-trifluoromethyl-benzyloxy)-benzylamino]-pyrrolidin-2-one	14.6
(S)-3-[3-Methyl-4-((E)-3-phenyl-allyloxy)-benzylamino]-pyrrolidin-2-one	11.0
(S)-3-[3-Methyl-4-(4-trifluoromethyl-benzyloxy)-benzylamino]-pyrrolidin-2-one	9.0
(S)-3-[3-Bromo-4-(4-trifluoromethyl-benzyloxy)-benzylamino]-pyrrolidin-2-one	12.6
(S)-3-[3-Methoxy-4-(naphthalen-1-ylmethoxy)-benzylamino]-pyrrolidin-2-one	9.6
(S)-3-[3-Methoxy-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	2.8
(S)-1-[3-Bromo-4-(2-fluoro-benzyloxy)-5-methoxy-benzyl]-pyrrolidine-2-carboxylic acid amide	10.9
Ralfinamide	23.0

I was also requested to carry our a study on inhibition of the sodium channels subtype Nav 1.3 by some representative compounds of the invention as per re; the results of the comparison with the reference standard ralfinamide are shown in Table 2.

For the "Electrophysiological assay" see Example 11 of the specification.

Table 2

Compounds	Nav 1.3 dep IC 50 μM
(S)-3-[3-Methyl-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	97
(S)-3-[3-Bromo-4-(3,5-dimethoxy-benzyloxy)-benzylamino]-pyrrolidin-2-one	169
(S)-3-(2-Chloro-4-pentyloxy-benzylamino)-pyrrolidin-2-one	119
(S)-3-[2-Chloro-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	118
(S)-3-[3,5-Dimethoxy-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	197
(S)-3-(3,5-Dimethyl-4-pentyloxy-benzylamino)-pyrrolidin-2-one	136
(S)-3-[3,5-Dimethyl-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	36
(S)-3-[4-(3,5-Dimethoxy-benzyloxy)-3,5-dimethyl-benzylamino]-pyrrolidin-2-one	187
(S)-3-[3,5-Dimethyl-4-(2-phenoxy-ethoxy)-benzylamino]-pyrrolidin-2-one	114
(S)-3-(4-Benzyloxy-3-methyl-benzylamino)-pyrrolidin-2-one	201
(S)-3-(3-Methyl-4-pentyloxy-benzylamino)-pyrrolidin-2-one	129
(S)-3-[3-Methyl-4-(4-trifluoromethyl-benzyloxy)-benzylamino]-pyrrolidin-2-one	143
(S)-3-[4-(3,5-Dimethoxy-benzyloxy)-3-methyl-benzylamino]-pyrrolidin-2-one	162
(S)-3-[3-Methoxy-4-(naphthalen-1-ylmethoxy)-benzylamino]-pyrrolidin-2-one	135
(S)-3-[3-Methoxy-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	109
(S)-2-[3-(2-Fluoro-benzyloxy)-benzylamino]-succinamide	156
N-{2-[3-Fluoro-4-(2-fluoro-benzyloxy)-benzylamino]-ethyl}-acetamide	182
(S)-1-[3-Bromo-4-(2-fluoro-benzyloxy)-5-methoxy-benzyl]-pyrrolidine-2-carboxylic acid amide	194
ralfinamide	202

The compounds shown a concentration and voltage dependent inhibition of the Nav 1.3 channels expressed in Xenopus oocytes. These results were predictive of a good analgesic potential in *in vivo* pain models.

For *in vivo* results, I carried out a "Formalin assay in mice" (Pain model) in which some compounds of the invention were given 20 mg/kg ip and 40 mg/kg po, 5 minutes before formalin injection; lamotrigine (ref. standard) was administered 20 mg/kg ip 15 minutes before formalin. The late phase (20-40 minutes after formalin injection) licking time was recorded. The data shown in the following Tabel 3 represent the mean % of the reduction of the licking time during the late phase versus control group (n=10 mice per group).

All compounds, administered either ip or po, showed a very good analgesic

activity.

Table 3

Compound	**% of inhibition licking time in the late phase at 20mg/kg ip**	**% of inhibition licking time in the late phase at 40mg/kg po**
(S)-3-[3-Methyl-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	83 ± 11.9	
(S)-3-(3,5-Dimethyl-4-pentyloxy-benzylamino)-pyrrolidin-2-one	65.4 ± 11.2	96.6 ± 2.6
(S)-3-[3-Methoxy-4-(5-phenyl-pentyloxy)-benzylamino]-pyrrolidin-2-one	79 ± 11.4	83.4 ± 8.3
Lamotrigine	83.8 ± 5.3	

Finally, I carried out a study on "Chronic Constriction Injury", a rodent model of neuropathic pain. Male Wistar rats weighing 200-225g were used for induction of neuropathic pain by a ligature of sciatic nerve performed as described by Bennett et al. (Pain 1988; 33:87-107). Seven days after surgery, mechanical allodynia was evaluated by Von Frey filaments following the method of Chaplan et al. (J Neurosci Method. 1994; 53:55-63). Animals that develop a paw withdrawal threshold in response to a Von Frey filament stimulus, corresponding to a pressure $\leq$ 5 g, were considered allodynic and included into the treatment groups.

Animals in the treatment groups were orally treated with 3-(4-Phenylpentoxy-3,5-dimethyl-benxylamino)-pyrrolidin-2-one at 10 and 30 mg/Kg, while animals in the control group were treated with vehicle.

The analgesic effect was evaluated as the increase of the withdrawal threshold in response to the mechanical stimuli and expressed as % of the Maximal Possible Effect (MPE) as shown in Table 4.

Table 4

Compound	%MPE	
(S)-3-(3,5-Dimethyl-4-pentyloxy-benzylamino)-pyrrolidin-2-one	30 min	60 min
10 mg/kg po	16.87	41.13
30 mg/kg po	19.26	49.6

This compound produced a dose and time dependent effect reaching about 50% of the MPE at 60' after the oral administration.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

MARCH 3, 2009

(date)

(signature)